behaviour and very large increase in the viscosities in all the range of shear rate.
**Conclusions:** When HA formulations were added to OA SF, the dynamic rheology demonstrated a gel-like behaviour for the cross-linked HA viscosupplement addition but that of a viscous solution for the non cross-linked HA addition.

## 449 STABILITY OVER TIME OF OSTEOARTHRITIC SYNOVIAL FLUID RHEOLOGY BEFORE AND AFTER ADDITION OF DIFFERENT HYALURONIC ACID FORMULATIONS

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**Purpose:** To evaluate, in vitro, the rheological stability over time, of the osteoarthritic (OA) synovial fluid (SF) before and after addition of 2 formulations of viscosupplements, a non cross-linked and a cross-linked hyaluronic acid (HA).
**Methods:** SF were obtained by sterile aspiration of the affected joints in 14 patients suffering from knee OA. SF samples were collected in sterile tubes and stocked at 4ºC. SF volume was recorded. Two viscosupplements (a cross-linked HA with an apparent MW of 6 mDa and a linear HA with an average MWof 1.1 mDa) were then added to SF in a ratio 1/1 to test their effects on SF behaviour. The rheological behaviour of SF and HA-modified-SF was determined using an AR 1000 rheometer. The rheological stability of SF and HA-modified-SF was studied over a 6 week period of time.
**Results:** In steady state flow the rheological behaviour of SF was non Newtonian. A slight decrease of the rheological behaviour appeared in the first time of aging and then stabilized. The addition of linear HA modified only slightly the behaviour of SF. It especially increased viscosity at higher shear rate and decreased shear rate dependence of the SF. On the opposite the addition of cross-linked HA induced a gel-like behaviour of the SF in all the range of frequency covered. No significant change was found at any time during the follow-up in both linear HA and cross-linked treated SF up of SF for both linear and cross-linked HA treated SF.
**Conclusions:** The stability of SF rheological behaviour over time after addition of viscosupplement suggests that, in vitro, the enzymatic degradation of exogenous HA is of negligible importance.

## 450 MECHANICAL STRESS-INDUCED CHRONOLOGICAL PHENOTYPIC CHANGES OF CHONDROCYTES IN THREE-DIMENSIONAL SCAFFOLD

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**Purpose:** Mechanical stress is a key regulator to the cell-activity of chondrocytes. Our major interest is focused on response of chondrocytes to mechanical stress. We investigated the chronological phenotypic changes of the three-dimensional (3D)-embedded chondrocytes *via* applying mechanical stress.
**Methods:** Freshly isolated chondrocytes from rat articular cartilage were cultured in monolayer. On reaching confluence, the cells were embedded in type I collagen gel, and pre-incubated for 24 h. The 3D-embedding cells were stimulated by mechanical stress for 1 h immediately after the 24 h-preincubation and were additionally incubated for 24 h after mechanical stimulation. The mechanical stress application was a cyclic loading at 5% strain, 0.33 Hz. Aggrecan (AGC) and type II collagen (Col2) mRNA expression was evaluated at 0 (served as control; t = 0), 1, 7, 13, and 25 h after the initiation of mechanical stimulation (n = 7). The total RNA was extracted from the 3D-embedding cells at the each time point for real-time RT-PCR. Real-time PCR was performed for AGC, Col2, and GAPDH, house-keeping gene, as an internal control. Statistical differences were determined by one-factor analysis of variance with Bonferroni/Dunn *post hoc* tests. *P* values <0.05 were considered significant.
**Results:** Mechanical stress clearly stimulated the 3D-embedded chondrocytes. Their ability to produce ECM molecules was measured at mRNA levels by real-time RT-PCR, and it was strongly and rapidly activated immediately after mechanical stimulation. Thus, at t = 1, both AGC ($P < 0.0001$) and Col2 ($P < 0.001$) expression was significantly increased between all the groups. However, at t = 7, 13, 25, AGC expression was significantly decreased compared to control ($P < 0.05$). Moreover, at t = 7, 25, Col2 expression was also significantly decreased compared to control ($P < 0.0001$).
**Conclusions:** In summary, the cell-differentiation of chondrocytes was strongly and rapidly enhanced immediately after mechanical stimulation. However, after that, it was rapidly down-regulated along the time. This study mainly provides that mechanical stimulation can play a key role in the chronological phenotypic change of chondrocytes in 3D scaffold.

## 451 DYNAMIC COMPRESSION COUNTERACTS FIBRONECTIN-FRAGMENT INDUCED INOS AND COX-2 EXPRESSION IN CHONDROCYTE/AGAROSE CONSTRUCTS

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**Purpose:** Osteoarthritis is a notoriously complex disease and there are likely to be multiple pathways underlying its cause and determining its progression. Thus, identifying which signals are activated during the different stages of the disease process, are under considerable investigation. Cartilage integrity involves both molecular and mechanical factors influenced by matrix degradation products such as the fibronectin fragments (FN-fs). Both the FN-fs and mechanical loading are known to influence the catabolic and anabolic processes, possible mediated by •NO release. It is plausible that mechanical loading may compete with the pathways activated by the FN-fs and promote subsequent downregulation of the catabolic signals. To date, no study has examined whether the pathways activated by the FN-fs will be influenced by dynamic compression. The present study examines the effects of the $NH_2$-hep I or COOH-hep II FN-fs on iNOS and COX-2 expression and production of •NO and $PGE_2$ release in chondrocytes cultured in agarose constructs and subjected to dynamic compression.
**Methods:** Chondrocyte/agarose constructs were subjected to dynamic compression (15%, 1 Hz frequency) in DMEM + 1xITS supplemented with 0 or 5 $\mu g \times ml^{-1}$ $NH_2$-hep I or 10 $\mu g \times ml^{-1}$ COOH-hep II FN-f and/or 1 mM 1400 W for 1, 6 or 48 hours. Real-time quantitative PCR assays coupled with molecular beacons were performed with cDNA, Brilliant® QRT-PCR Master Mix, primer pairs and analysed on the MX3000P QPCR instrument. The ratio of the relative expression levels of the catabolic (iNOS, COX-2) and anabolic (aggrecan, collagen type II) signals were accomplished by normalizing each target to the reference gene, GAPDH and to the calibrator sample, by a comparative cycle threshold approach. Nitrite and $PGE_2$ release were measured in the media by Griess and EIA assay. $[^3H]$-thymidine and $^{35}SO_4$ incorporation were measured by TCA and alcian blue precipitation, respectively.
**Results:** The $NH_2$-hep I or COOH-hep II FN-fs induced the relative expression levels of iNOS and COX-2 and increased the production of •NO release. Both fragments downregulated aggrecan and collagen type II expression levels and subsequent inhibition of $[^3H]$-thymidine and $^{35}SO_4$ incorporation. The FN-f-induced effects on iNOS, COX-2 aggrecan and collagen type II expression could be reversed with the application of dynamic compression in the presence of the iNOS inhibitor, 1400 W (Fig. 1). In addition, co-stimulation with dynamic compression and iNOS inhibitor inhibited nitrite release and upregulated $[^3H]$-thymidine and $^{35}SO_4$ incorporation in the presence of the fragments. In contrast, $PGE_2$ levels were not significantly influenced by the presence of the $NH_2$-hep I or COOH-hep II FN-f and/or dynamic compression.